ONLINE INQUIRY & INVESTIGATION

DEGRADATIVE ENZYMES from the Pharmacy or Health Food Store: Interesting Examples for Introductory Biology Laboratories

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Enzymes are molecular catalysts that increase the rates of chemical reactions in living cells by facilitating the conversion of a specific substrate to a particular product (Walsh, 2001). They play an essential role in the conversion of light or chemical bond energy into ATP, in the transformation of nutrients containing carbon or nitrogen into usable metabolites, in the replication and expression of genetic information, and in the detection and transduction of external chemical signals. Most enzymes are proteins, although there are now a number of important examples of catalytic RNAs or ribozymes. Among the key properties of an enzyme-catalyzed reaction are the dependence of the reaction rate on both the enzyme and substrate concentration, the specificity of the enzyme for its substrate, the sensitivity of the enzyme to high temperatures or extreme pH, and the regulation of the enzyme's activity by other compounds that may serve as inhibitors or activators.

Because of the importance of enzymes, almost all science curricula include a discussion of the general properties of enzymes and at least one laboratory experiment in which the activity of a particular enzyme is investigated. For students in grades K-12, the material on enzymes may be presented to meet the Life Science Content Standards (National Research Council, 1996) for Levels 5-8 (Structure and function in living systems) or Levels 9-12 (Matter, energy, and organization in living systems). For college or university students, the material on enzymes may be presented in the section of an introductory biology course for majors or nonmajors which deals with cell structure and function. A survey of 10 currently-available biology laboratory manuals for college students reveals that most of them use the same enzymes to illustrate the basic principles of enzyme activity (Table 1). These enzymes include:

- α-amylase, which catalyzes the degradation of starch to simple sugars
- catalase, which catalyses the conversion of hydrogen peroxide to oxygen and water
- catechol oxidase, which catalyzes the oxidation of catechol to benzoquinone. This last enzyme also has been called catecholase, tyrosinase, and polyphenol oxidase.

While these particular enzymes are readily available and their activity can be demonstrated at relatively low cost, they do not necessarily produce good numerical data. As a result, students may find experiments with them frustrating, and they may be unable to carry out more advanced studies such as those involving enzyme kinetics. In this article, I describe procedures for measuring the activities of several degradative enzymes that are readily available in over-the-counter products in pharmacies or health food stores. The properties of these enzymes can be studied using simple colorimetric assays with relatively inexpensive synthetic substrates. While these enzymes can be studied qualitatively as a visual increase in color intensity, one of their advantages is that they have very good quantitative characteristics. That is, when the amount of product is measured as an absorbance value in a simple spectrophotometer, these enzymes yield excellent numerical data that can be graphed and analyzed. Because these degradative enzymes are used to treat health-related conditions with which most students are familiar, they may be of greater interest than enzymes from potatoes or other sources. In this paper, I first describe the experimental analysis of the enzyme β-galactosidase or lactase from Lactaid™ pills in some detail as one example of a degradative enzyme. I then summarize similar procedures for measuring the activities of several other degradative enzymes. Finally, I discuss some alternative ways of using these enzymes in different laboratory settings.

β-Galactosidase Activity from Lactaid™ Pills

Lactose, normally found in milk and other dairy products, is a disaccharide composed of D-glucose and D-galactose joined together through a β(1-->4) glycosidic bond. It can be degraded

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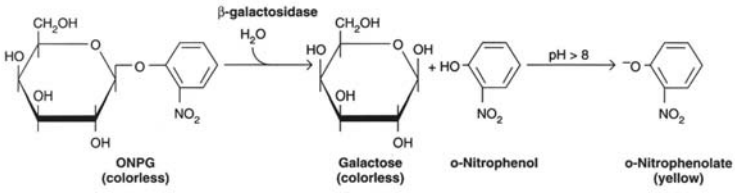
Table 1. Enzymes Used in Current General Biology Laboratory Manuals.

LAB MANUAL	ENZYME	SUBSTRATE	PRODUCT	METHOD*
Abramoff, P. & Thomson, R. G. (1994) *Laboratory Outlines in Biology VI* W. H. Freeman	amylase	starch	hydrolyzed starch	quantitative: decrease in I_2 binding (A_{560})
	starch phosphorylase	glucose-1-phosphate	starch	qualitative: increase in I_2 binding
Bres, M. & Weisshaar, A. (2005) *Thinking about Biology: an introductory laboratory manual* (2/e) Pearson/Prentice-Hall	amylase	starch	simple sugars	qualitative: I_2 binding to starch, reaction of sugars with Benedict's reagent
Dickey, J. (2003) *Laboratory Investigations for Biology* (2/e) Pearson/ Benjamin Cummings	catechol oxidase	catechol	benzoquinone	quantitative: increase in A_{540}
Enger, E. D. & Ross, F. C. (2005) *Laboratory Manual for Concepts in Biology* (11/e) McGraw Hill	catechol oxidase	catechol	benzoquinone	qualitative: increase in color
Gunstream, S. E. (2005) *Explorations in Basic Biology* (10/e) Pearson/Prentice-Hall	catalase	hydrogen peroxide	oxygen	quantitative: gas formation (increase in foam thickness)
Helms, D. R., Helms, C. W., Kosinski, R. J. & Cummings, J. R. (1998) *Biology in the Laboratory* (3/e) W. H. Freeman	catechol oxidase	catechol	benzoquinone	quantitative: increase in A_{420}
Mader, S. S. (2004) *Laboratory Manual for Biology* (8/e) McGraw Hill	catalase	hydrogen peroxide	oxygen	quantitative: height of bubble column
Morgan, J. G. & Carter, M. E. B. (2005) *Investigating Biology* (5/e) Pearson/Benjamin Cummings	catechol oxidase	catechol	benzoquinone	qualitative: increase in color
	amylase	starch	hydrolyzed starch	quantitative: time to loss of I_2 binding
Perry, J. W., Morton, D. & Perry, J. B. (1995) *Laboratory Manual for Starr and Taggart's Biology: The Unity and Diversity of Life* and *Starr's Biology: Concepts and Applications* Thomson Learning/Brooks/Cole	catechol oxidase	catechol	benzoquinone	qualitative or quantitative: increase in A_{540}
Vodopich, D. S. & Moore, R. (2005) *Biology Laboratory Manual* (7/e) McGraw Hill	catechol oxidase	catechol	benzoquinone	qualitative: increase in color
	catalase	hydrogen peroxide	oxygen	quantitative: height of gas column
	peroxidase	guaiacol	oxidized guaiacol	quantitative: increase in A_{470}

* Qualitative methods are those that are based on a visual estimation of color intensity or some other measurement of enzyme activity. Quantitative methods are those that involve measurement of absorbance values at a particular wavelength in a spectrophotometer.

into its component sugars in the small intestine by an enzyme called β-galactosidase or lactase. Most persons of Northern European origin have adequate levels of this enzyme as adults and so have no trouble digesting milk products. However, people from other parts of the world often have a condition called lactose intolerance (Swagerty et al., 2002). These individuals have high levels of β-galactosidase at birth and so can digest the lactose in breast milk. However, they gradually produce less enzyme as they grow older and so cannot digest lactose as adults. If they do consume milk, yogurt, ice cream, or other dairy products, the lactose accumulates in the small intestine, resulting in diarrhea, gas formation, and the associated symptoms of bloating, cramping, and flatulence. There are several over-the-counter products on the market such as Lactaid™ that can be used to treat lactose intolerance. In these preparations, the enzyme β-galactosidase is combined with other ingredients such as microcrystalline cellulose, calcium carboxymethylcellulose, dextrose, sodium citrate, silicon dioxide, and magnesium stearate, and pressed into tablets or caplets. The exact amounts and functions of the additional ingredients are not stated, but they are usually included to protect the enzyme protein as it moves through the digestive system.

How can one measure the activity of this β-galactosidase? The protein itself does not exhibit any unique light absorption or fluorescence and neither do the natural substrate (lactose) and products (galactose and glucose) of the chemical reaction. However, β-galactosidase can hydrolyze several synthetic substrates, creating products that are easy to detect. The most commonly-used of these compounds is o-nitrophenyl-β-D-galactopyranoside (ONPG). ONPG consists of D-galactose and o-nitrophenol (also called 2-nitrophenol), joined through a β-linkage similar to that found in lactose. Hydrolysis of this bond by β-galactosidase results in the formation of free D-galactose and o-nitrophenol. D-galactose is again difficult to detect, but o-nitrophenol has a distinct yellow color. At pH 7, which is normally optimal for β-galactosidase activity, o-nitrophenol has a pale yellow color. However, at more alkaline pH (>8), the hydroxyl group in o-nitrophenol loses its hydrogen (deprotonates) to form the o-nitrophenolate anion that has a more intense yellow color. See the reaction below.



The use of ONPG as a substrate makes the measurement of β-galactosidase or lactase activity particularly simple. A solution containing the enzyme is usually combined with a buffer and other salts designed to keep the pH constant at pH 7.0. The substrate (ONPG) then is added and the solution is incubated at a certain temperature. As ONPG is broken down and o-nitrophenol is formed, a pale yellow color develops. The reaction then is stopped by adding a basic solution such as 1.0 M sodium carbonate (Na_2CO_3). This raises the pH of the reaction to about pH 10, where the enzyme no longer functions and where the color intensifies. The exact time interval between when the substrate was added and when the reaction was stopped is noted. The yellow color then can be estimated qualitatively by eye or measured quantitatively in a spectrophotometer. In the latter case, the absorbance of the solution then can be converted to an exact amount of the product in nmoles or μmoles, and the β-galactosidase activity can be expressed as the number of nmoles or μmoles of product formed per minute.

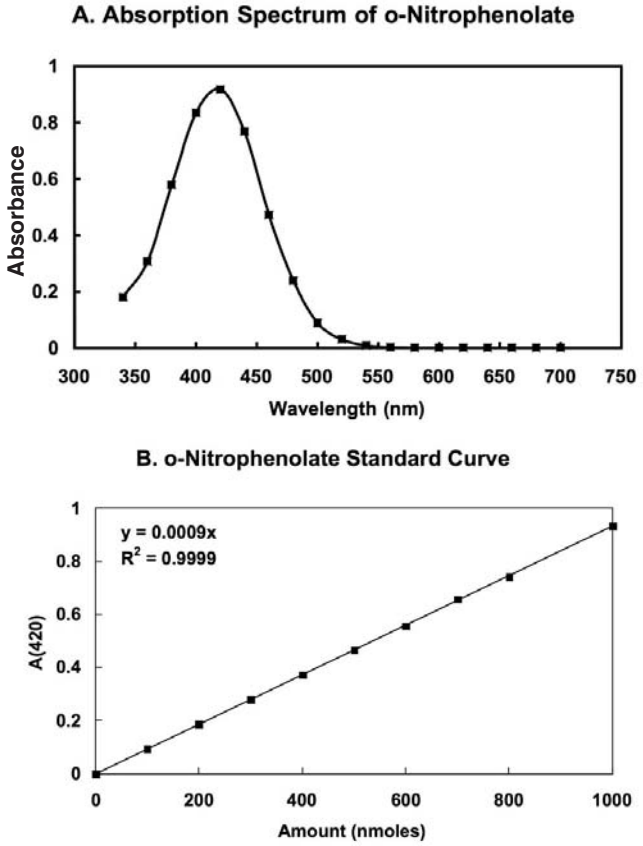
Experimental Procedures for Measuring β-Galactosidase Activity

Measurement of the β-galactosidase activity in Lactaid™ tablets usually requires preparation of an extract. I normally have students grind individual tablets in a mortar with a pestle and suspend the powder in 5 ml of buffer. I usually use Z buffer (Miller, 1972), a phosphate-based buffer at pH 7.0 that contains small amounts of KCl, $MgSO_4$, and 2-mercaptoethanol. However, because some students find the smell of the 2-mercaptoethanol offensive, the experiment can also be done simply with 0.1 M potassium phosphate buffer, pH 7.0. After 15 minutes, the suspension is centrifuged in a clinical centrifuge (3000 rpm for five minutes) to remove the insoluble material. The liquid supernatant is saved as the working enzyme solution. Lactaid™ is also available in a liquid form and it is possible simply to dilute this product into the buffer solution.

To measure the β-galactosidase activity in the initial extract or solution quantitatively, it is necessary to know the wavelength of light at which the absorbance of the o-nitrophenolate anion is maximal and to construct a standard curve. I have students set up a series of 13 x 100 mm tubes containing 3.5 ml of buffer, 0.5 ml 1.0 M sodium carbonate (106 g/liter), and varying amounts of water and 1.0 mM o-nitrophenol (13.9 mg/100 ml) to yield a total volume of 5.0 ml. The absorbance of one of the yellow solutions then is measured at 20 nm intervals in a spectrophotometer from 340 nm to 700 nm. Typical results are shown in Figure 1A. I currently use Thermo Spectronic Genesys 20 instruments and standard plastic cuvettes. However, any colorimeter will work, and with most instruments, the 13 x 100 mm tubes with the o-nitrophenolate solutions will fit directly into the cuvette holder. Once the wavelength of maximal absorbance has been determined, the absorbance of each of the individual tubes is measured. The absorbance of each tube then is plotted as a function of the amount of o-nitrophenolate it contains to make a standard curve. A typical standard curve at 420 nm is shown in Figure 1B. From this graph, a conversion factor related to the slope of the line is created that will allow any unknown absorbance within the linear range of the standard curve to be converted to a specific amount of o-nitrophenolate.

Because the amount of β-galactosidase activity with ONPG as the substrate in the Lactaid™ extract is unknown, I normally have the students make four serial 1/10 dilutions in buffer and carry out a preliminary assay to find a suitable dilution to use for later experiments. A 0.5 ml aliquot of each dilution is combined with 3.5 ml of Z buffer in a 13 x 100 mm tube, and 0.5 ml of 10 mM ONPG (3 mg/ml, Sigma Aldrich N1127, $23.50/g) is added to start the reactions. Because it is important to know the exact time at which the substrate is added, I have the students add the substrate to the tubes at 30-second intervals and rapidly mix the solutions by covering the tubes with Parafilm™ and inverting them several times. The students then can watch the color development in the tubes by eye as they incubate at room temperature. When a pale yellow color is visibly apparent, 0.5 ml of 1.0 M sodium carbonate is added to stop the reaction and the solution is mixed by inversion again. There is a significant increase in color intensity when the base is added, so it is important not to let the reactions get too dark. As expected, the tubes with the more concentrated

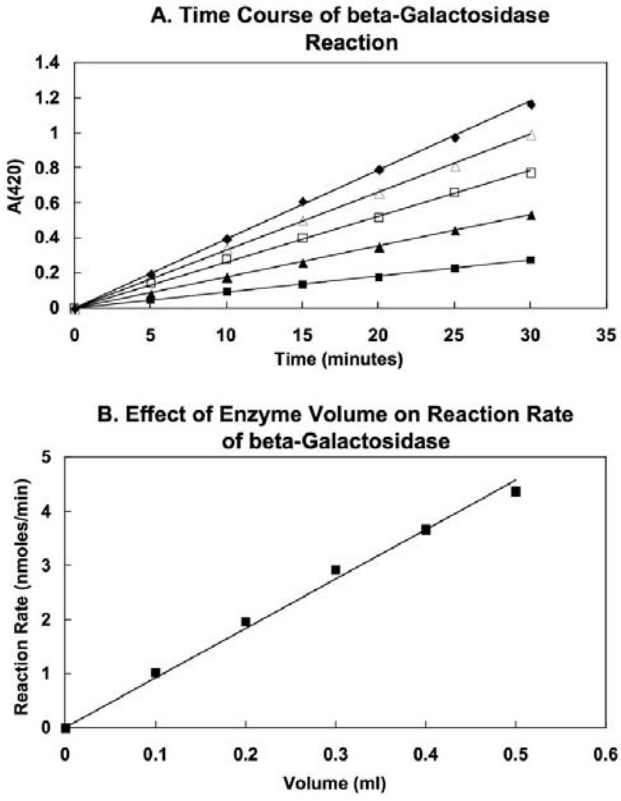
Figure 1. Measurement of o-nitrophenolate formed by hydrolysis of o-nitrophenyl-β-D-galactopyranoside by β-galactosidase from Lactaid™. Panel A shows the absorption spectrum of the o-nitrophenolate anion from 400-700 nm. Panel B shows an o-nitrophenolate standard curve at 420 nm.



enzyme solutions turn yellow first. The absorbance of each of the reactions then is measured in the spectrophotometer and the amount of product in nmoles determined from the standard curve. By dividing the amount of product by the time interval during which the reaction occurred, the activity of the enzyme in nmoles/min can be determined. Once a suitable dilution has been identified (i.e., one which gives a measurable absorbance value within the linear range of the standard curve in a reasonable period of time), I have the students do three replicate assays with that solution so they can calculate an average rate reflecting the reproducibility of the assay.

One of the advantages of using β-galactosidase as a model enzyme is that it has very good quantitative characteristics. Figure 2A shows the time course of the β-galactosidase reaction using different volumes of a 10^{-3} dilution of a stock Lactaid™ solution. In this case, individual reactions were intentionally stopped at five-minute intervals so progress of the reaction could be demonstrated. It is clear that product formation is linear with time, so that stopping the reactions on the basis of visual color development is experimentally valid. Figure 2B shows that when the rate of the reaction in nmoles/min is plotted as a function of the enzyme volume, there is again a simple linear relationship. I have found that some students prefer to stop the preliminary set of reactions at fixed-time intervals and I let them do this. However, most eventually become comfortable with the visual color development method because it is easier.

Figure 2. Properties of the β-galactosidase activity from Lactaid™. Panel A shows the time course of the reaction with 0.5 ml (◆), 0.4 ml (△), 0.3 ml (□), 0.2 ml (▲), and 0.1 ml (■) of a 10^{-3} dilution of a stock enzyme extract. Panel B shows the relationship between the reaction rate in nmoles/min and the enzyme volume.



Properties of β-Galactosidase Activity from Lactaid™ Pills

Once students have learned to measure the β-Galactosidase activity from Lactaid™ pills, they can carry out a variety of experiments to explore the properties of the enzyme. One important characteristic of any enzyme is its substrate specificity. I have had students determine the specificity of the β-galactosidase reaction by using a series of similar synthetic substrates in the standard assay method. These include o-nitrophenyl-α-D-galactopyranoside, o-nitrophenyl-β-D-glucopyranoside, p-nitrophenyl-β-D-galactopyranoside, p-nitrophenyl-α-D-galactopyranoside, p-nitrophenyl-α-D-mannopyranoside, and so on. It turns out that the β-galactosidase from Lactaid™ is absolutely specific for the presence of D-galactose in a β linkage and only p-nitrophenyl-β-D-galactopyranoside is hydrolyzed at any appreciable rate.

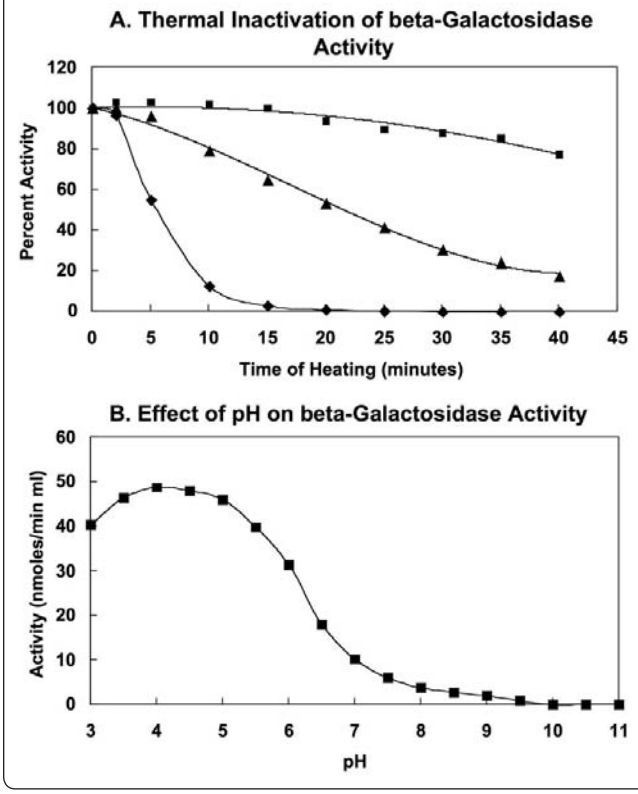
I also have had students assess the effects of temperature and pH on enzyme activity with ONPG as the substrate. The effect of temperature can be determined in two ways. One way is by changing the temperature at which the reaction is run using a series of water baths. The reaction rate gradually increases, but it then drops to zero when temperatures that cause denaturation of the protein are reached. Another way is by heating a solution containing the enzyme at a particular temperature in a water bath, removing samples at different times, and then assaying the samples for enzyme activity at room temperature. Figure 3A shows the results of this type of experiment. One of the

advantages of this experimental approach is that it separates the denaturation effects of high temperature on the enzyme protein from kinetic effects of heat on enzyme-substrate collisions.

The effect of pH on the reaction can be determined by changing the pH of the buffer in which the reaction is run. I have used a series of Z or phosphate buffers in which the pH has been adjusted with either HCl or KOH. With most purified enzymes, a single symmetrical peak of activity is usually observed with maximal activity at the optimum pH. Figure 3B shows the effect of pH on the β-galactosidase activity from Lactaid™ pills. In this case, while activity falls off at alkaline pH, it actually increases at acidic pH. While this result might appear surprising, it is consistent with the fact that Lactaid caplets containing β-galactosidase must pass through the stomach at pH 2 before reaching the small intestine.

I also have had students study the kinetics properties of β-galactosidase by varying the amount of ONPG in the standard reaction mixture. The enzyme shows simple Michaelis-Menten kinetics. As shown in Figure 4A, the reaction rate increases with the substrate concentration and eventually reaches a maximum velocity (V_{max}). When the data are analyzed in a linear Lineweaver-Burke plot, a simple straight line is produced that can be used to estimate both V_{max} and the Michaelis constant or K_m (Figure 4B). Once these basic kinetic parameters have been determined, students can study the effects of activators or inhibitors. When the activity of β-galactosidase is measured with ONPG as the substrate in the presence of various monosaccharides and disaccharides, only D-galactose causes significant inhibition. Kinetic analysis indicates, not surprisingly, that D-galactose is a competitive inhibitor that binds to the active site.

Figure 3. Effects of temperature and pH on the β-galactosidase activity from Lactaid™. Panel A shows the effects of heating a 10^{-3} dilution of a stock enzyme extract at 50° C (■), 55° C (▲), and 60° C (◆) for varying periods of time. The samples were chilled on ice and then tested for activity at room temperature. Panel B shows the effects of measuring the β-galactosidase activity in a 10^{-3} dilution of a stock enzyme extract in Z buffer adjusted to different pHs.



Measurement of the Activities of Other Degradative Enzymes

Several other over-the-counter products contain degradative enzymes that can be measured using essentially the same methods. One convenient example is Beano®, a product that is designed to reduce the gas and flatulence associated with beans and other legumes. It contains the enzyme α-galactosidase and again is available in both a tablet and liquid form. The activity of this enzyme can be measured in reactions containing 3.5 ml of buffer, 0.5 ml of an appropriately diluted enzyme solution, and 0.5 ml of 10 mM p-nitrophenyl-α-D-galactopyranoside (3 mg/ml, Sigma Aldrich N0877, $57.50/g). The reactions are again stopped with 0.5 ml of 1.0 M sodium carbonate. The amount of p-nitrophenol is determined by measuring the absorbance at 410 nm. As shown in Figure 5A, the rate of product formation with different volumes of a 10^{-3} dilution of a Beano® extract again is linear with time and proportional to the amount of enzyme in the reaction.

Other useful examples are products designed to promote healthy digestion. These tablets or capsules often contain a mixture of degradative enzymes, including α-amylase for the breakdown of starch, bromelain, papain, pepsin, or other proteases for

Figure 4. Kinetics of the β-galactosidase activity from Lactaid™. Panel A shows the rate of the reaction in the presence of varying amounts of the substrate ONPG. Panel B shows a Lineweaver-Burke plot of the same data in which 1/Reaction Rate is plotted as a function of 1/[S].

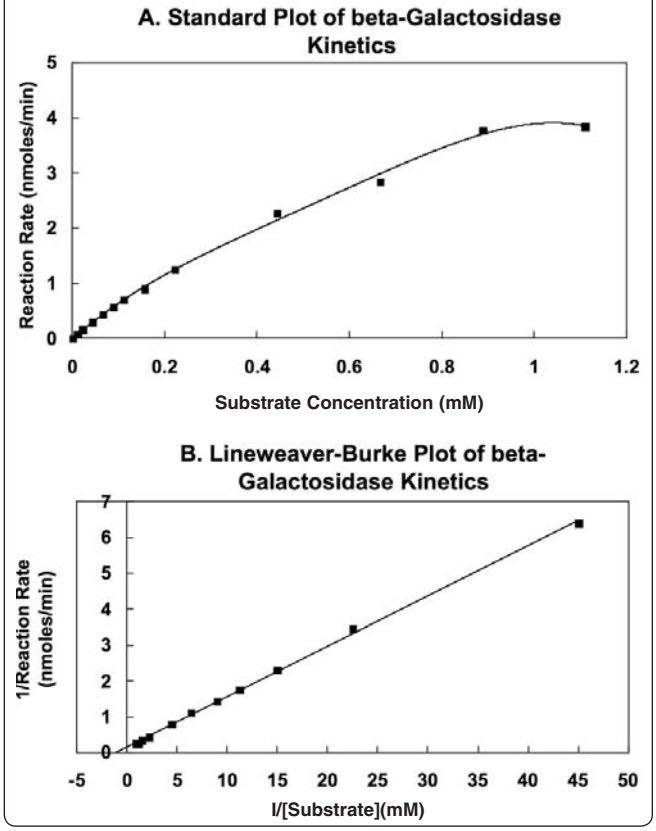
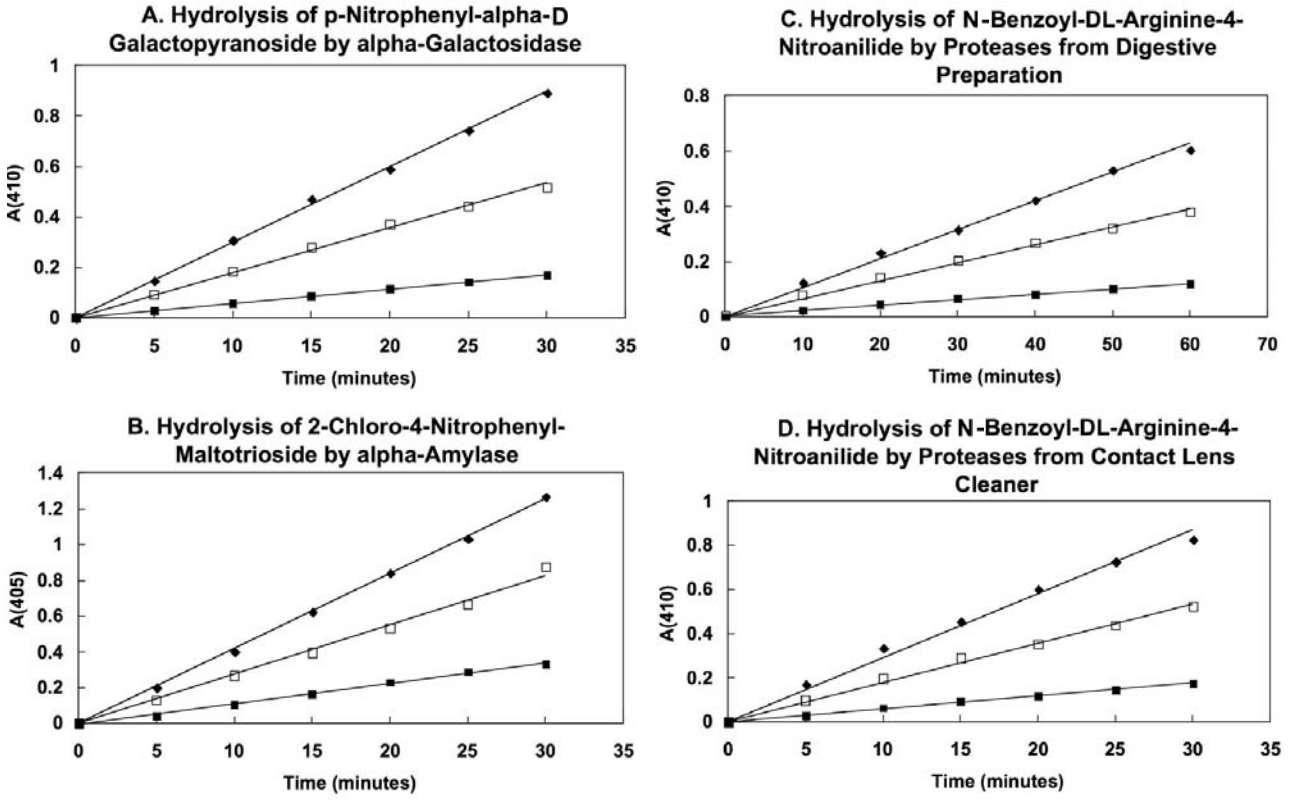


Figure 5. Measurement of the activities of other degradative enzymes. Panel A shows the time course of the α-galactosidase reaction with 0.5 ml (◆), 0.3 ml (□), and 0.1 ml (■) of a 10^{-3} dilution of a stock extract of Beano® and p-nitrophenyl-α-D-galactopyranoside as the substrate. Panel B shows the time course of the α-amylase reaction with 0.5 ml (◆), 0.3 ml (□), and 0.1 ml (■) of a 10^{-2} dilution of a stock extract of Zyme-Aid® and 2-chloro-4-nitrophenyl-maltotrioside as the substrate. Panel C shows the time course of the protease reaction with 0.5 ml (◆), 0.3 ml (□), and 0.1 ml (■) of a 10^{-2} dilution of a stock extract of TwinLabs Pancreatin® and N-benzoyl-DL-arginine-4-nitroanilide as the substrate. Panel D shows the time course of the protease reaction with 0.5 ml (◆), 0.3 ml (□), and 0.1 ml (■) of a 10^{-1} dilution of Alcon Supra Clens® and N-benzoyl-DL-arginine-4-nitroanilide as the substrate.



the breakdown of proteins; and lipase for the breakdown of fats. The activity of the α-amylase can be measured using the synthetic substrate 2-chloro-4-nitrophenyl-maltotrioside. This compound is available from several sources (Genzyme Diagnostics, Pointe Scientific) as a part of a reagent used to measure α-amylase activity in clinical chemistry labs. As before, the reactions contain 3.5 ml of buffer, 0.5 ml of a suitable enzyme solution, and 0.5 ml of a 10 mM (6.6 mg/ml) substrate solution. After a visible color has developed, the reactions are stopped with 0.5 ml of 1.0 M sodium carbonate and the absorbance is measured at 405 nm. As shown in Figure 5B, the rate of product formation with a 10^{-2} dilution of a product called Zyme-Aid® shows good linearity both with time and the amount of enzyme.

The protease activity in these digestive aids can be measured in a similar way using the synthetic substrate N-benzoyl-DL-arginine-4-nitroanilide (4.3 mg/ml, Sigma Aldrich B4875, $26.00/g). Reactions containing 3.5 ml of buffer, 0.5 ml of a suitable enzyme solution, and 0.5 ml of 10 mM substrate are set up and allowed to develop color as before. In this case, the reactions are stopped by adding 0.5 ml of 1.0 M HCl to acidify the reaction. The color of the product (4-nitroaniline) does not increase when the reactions are stopped and the absorbance can be read at 410 nm. This substrate is acted on by several enzymes (trypsin, bromelain, and papain) and so what is measured in these reactions is the total amount of proteolytic activity with this compound. Figure 5C shows that the amount of product formed with a 10^{-2} dilution of a pancreatin extract is again proportional to time and enzyme volume.

Another example of an over-the-counter product that contains degradative enzymes is enzymatic contact lens cleaner. The protease activity in Alcon Supra Clens® can be measured with the same substrate (N-benzoyl-DL-arginine-4-nitroanilide) and protocol used with the digestive enzymes. In this case, the liquid cleaner is simply diluted into buffer and then tested for activity. As shown in Figure 5D, different volumes of a 10^{-1} dilution again give reasonable reaction rates, although there seems to be some deviation from linearity with time.

Technical Issues & Laboratory Safety

I have used different versions of these experiments with degradative enzymes in summer programs for minority studies, in introductory biology labs for majors or for nonmajors, and in more advanced labs in cell biology or biochemistry. Depending on the background of the students, the interests of the instructor, and the equipment available, studies of enzyme activity can be done either qualitatively or quantitatively. For qualitative studies, students could simply look for the presence or absence

of a visible yellow when the enzyme and substrate are combined or when the enzyme is exposed to various environmental conditions. For quantitative studies, students could use stopwatches to precisely measure intervals and spectrophotometers to determine actual absorbance values. I usually have the students use 5 or 10 ml pipets for larger volumes of solutions like buffers and micropipettors for smaller volumes of solutions like the enzyme extract and substrate. However, smaller volumes of liquids could be dispensed with 1 ml pipets if they were available. Instead of using pipets or micropipettors, the students could also add reagents drop-wise to the reaction mixtures (20 drops from a Pasteur or transfer pipet is about 1.0 ml).

Normal laboratory safety procedures (goggles, lab coats, closed-toed shoes, long pants) should be followed in carrying out these experiments. This is particularly important where reagents that are acidic or basic are involved. All liquids should be transferred either with micropipettors and disposable plastic tips or with glass or plastic pipets and Pipet-Aids or pumps. No mouth pipetting should be allowed. Because all of the reagents used in these experiments are simple aqueous solutions, we normally pool the liquid waste from the enzyme reaction mixtures into large bottles and then ship the bottles to an appropriate disposal site along with the rest of our chemical waste. The glassware used in the experiments is either washed or disposed of safely.

Alternative Protocols with Digestive Enzymes

Experiments using these degradative enzymes can be done as part of didactic laboratory exercises, as part of teacher-initiated lab projects, or as part of student-driven inquiry-based investigations. Depending on the situation, an instructor may wish to devote a single laboratory session to the study of one enzyme, to spend several consecutive periods on a project involving one enzyme, or to spread out the study of enzymes over a longer period of time and to intersperse work with several enzymes with other types of lab projects. For a single didactic session, it would probably be easiest to use a liquid source of enzyme and to tell the students which wavelength of light to use for the absorbance measurements. For a longer teacher-initiated project, it would be feasible to do the absorption spectrum and standard curve in first period, the basic assays of enzyme activity in the second period, and more advanced experiments in the third, fourth, or fifth periods. This is the approach I normally use, since it gives students time to develop their laboratory skills. Having learned how to measure an enzyme's activity accurately, they can then participate in the design of additional experiments to study its properties (Leonard, 1989; Sundberg & Moncada, 1994). The data obtained in experiments with these degradative enzymes can be easily used as the basis of poster or PowerPoint presentations and lab reports (McMillan, 2001; Mulnix & Penhale, 1997).

For student-driven inquiry experiments, a teacher may want to begin with a general discussion of enzymes but then give the students more opportunity to plan their own experiments. These experiments might include measuring activity of the brand-name and generic forms of a product such as Lactaid™, determining the activity of the enzymes in the Lactaid™ and Beano® preparations with different chemical substrates, or comparing the properties of the purified form of an enzyme such as β-galactosidase with that of the commercial product. Many of the commonly-available digestive aids use different methods for expressing their activity: Some refer to the amount of a proteolytic enzyme in mg but others use USP or FCC units. It is not obvious what these units mean and so determining the activity of several different products with a common substrate and expressing the activities in common units (nmoles/min ml) would be a useful project. Another approach might be to present the students with one or more extracts of over-the-counter preparations as unknowns. They might then determine which enzyme activities are present in these solutions using the different synthetic substrates. There is really no limit to what can be done with this useful experimental system.

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